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## Does a new polyomavirus contribute to Merkel cell carcinoma?

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### Abstract

A new technique designed to hunt for non-human transcripts has identified a novel SV40-like virus present in the majority of Merkel cell carcinomas. Here we examine what it will take to determine whether or not this virus contributes to carcinogenesis.

Merkel cell carcinoma (MCC) is an uncommon and aggressive skin cancer. More than one-third of MCC patients will die from this cancer, making it twice as lethal as malignant melanoma [1]. The reported incidence of MCC has tripled over the past 20 years because of improved detection and a rise in the number of elderly and immunosuppressed individuals who are at risk [2]. It is estimated that there will be 1,500 new cases of MCC this year in the United States [3].

There has been a long-standing interest in the etiology of MCC. Several lines of evidence suggest a strong link between MCC and ultraviolet light exposure. For example, the incidence of MCC is higher at more equatorial latitudes [4], 81% of primary tumors occur on sun-exposed skin [1], and Caucasians have the greatest risk. MCC incidence is also strongly coupled with advanced age; indeed, 90% of people diagnosed with MCC are over the age of 50 [1].

Importantly, there is a striking epidemiologic association between immunosuppression and MCC. Chronically immunosuppressed individuals are more than 15 times more likely to develop MCC than are age-matched controls [1]. In particular, many forms of T-lymphocyte immune suppression are linked with MCC: HIV infection [5], solid organ transplantation [6] and chronic lymphocytic leukemia [1] are all associated with a ten-fold or greater increased risk of developing MCC. MCC is more frequently lethal in immunosuppressed patients, with a reported disease-specific mortality of up to 56% [7]. Interestingly, there are several case

reports of MCC regression following restoration of immune function [8,9]. These intriguing clues have led many to suspect an infectious contribution to MCC.

### A new technique finds a new virus

Patrick Moore and Yuan Chang are well known for their discovery of the Kaposi's sarcoma-associated herpesvirus (KSHV) [10]. Nearly a decade ago, they became interested in MCC because of its strong links to immunosuppression. In a recent paper in *Science* (Feng *et al.* [11]) their team reports the development of a technique known as digital transcriptome subtraction (DTS; see [12]) and its use to look for viral RNA sequences in MCC cells. The authors first sequenced nearly the entire transcriptome of several MCC tumors using pyrosequencing technology [11]. Pyrosequencing is a massively high-throughput, bead-based chemiluminescent sequencing method that produces large numbers of medium-length sequences (typically 300 nucleotides) simultaneously. The resulting MCC cDNA sequences were filtered to eliminate low-complexity sequences, known human di- and trinucleotide repeat sequences, and poly(A) and poly(T) tracts. The remaining 'high-fidelity' sequences were compared to human transcripts in the RefSeq database using an *in silico* approach similar to that described by Weber *et al.* [13].

Feng *et al.* [11] obtained 382,747 high-fidelity MCC cDNA sequences, of which 99.4% aligned closely with known human transcripts. One sequence that did not align had high

sequence identity with the human BK polyomavirus T antigen. This transcript sequence was extended using rapid amplification of cDNA ends (RACE), and then used to develop bidirectional primers for viral genome sequencing. Several successive steps of genome walking led to the discovery of two nearly identical full-genome sequences of a novel human DNA polyomavirus, which the authors named Merkel cell polyomavirus (MCPyV or MCV; GenBank accession number EU375803, GenBank EU375804).

Feng *et al.* [11] screened ten MCC tumors for MCPyV and found that seven were strongly positive for MCPyV DNA by PCR and an additional tumor was weakly positive. The strength of the PCR signals raised the question of whether the viral DNA was integrated in a clonal fashion. Genomic DNA from MCC tumors was digested with two restriction enzymes and analyzed by Southern hybridization using a MCPyV DNA probe. From the observed banding patterns, five of the eight MCPyV-positive MCC tumors revealed monoclonal integration of the virus, another tumor had a monoclonally integrated viral concatemer, and two additional tumors had an indeterminate integration pattern. For one tumor with a monoclonal integration pattern, both primary and metastatic tumor tissue was available, and both specimens showed an identical viral integration pattern, suggesting that integration of MCPyV preceded MCC tumor metastasis. Furthermore, the distinct integration patterns between tumors imply that the virus integrated at different locations within the human genome in the different tumors. Although no direct assessment of viral RNA or protein expression was described, the DTS results indicated that at least one MCC tumor expressed viral RNA sequences corresponding to the large T antigen. As discussed below, polyomavirus large T antigens have proven transforming abilities in mammalian cells.

### Polyomaviruses and carcinogenesis

Polyomaviruses are a genus of non-enveloped viruses with a circular double-stranded DNA genome of approximately 5 kb. It has been well established that many polyomaviruses are able to transform mammalian cells [14]. The best-characterized polyomavirus, SV40, was originally discovered as a contaminant in the primary monkey kidney cells used to prepare early batches of the polio vaccine and was subsequently shown to induce tumors in newborn hamsters [15,16].

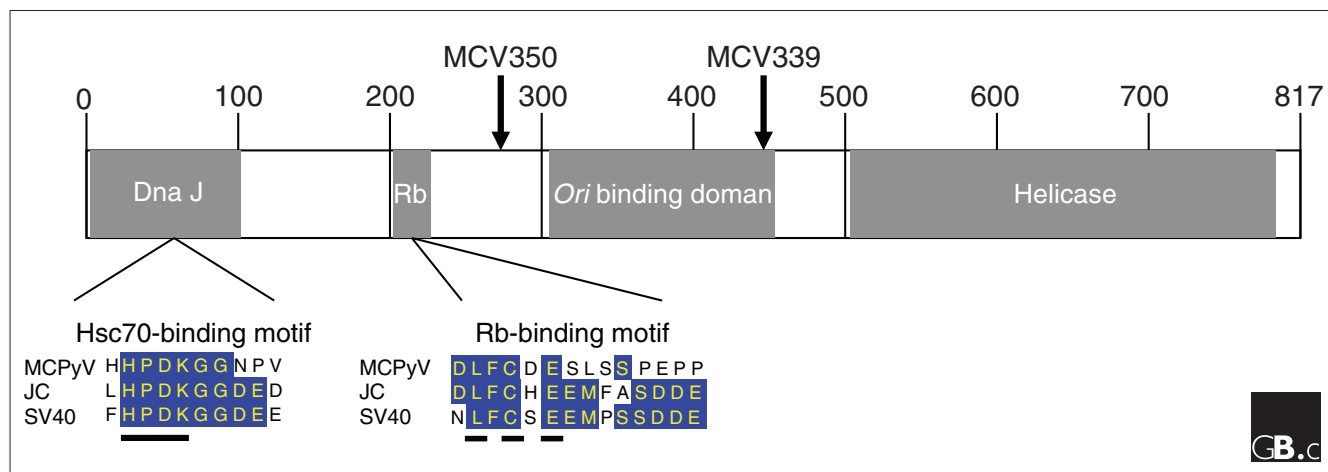
Polyomaviruses do not encode the entire repertoire of proteins necessary for viral DNA replication. Instead, these viruses hijack the machinery of a dividing host cell. Polyomaviruses express genes in two waves: early and late. The early-expressed genes, including large and small T antigens, bind to host proteins to force the cell into S phase (the cell-cycle phase when the DNA is replicated) and facilitate viral replication. The late genes encode components of the viral coat and enable lysis.

Large T antigen performs diverse functions that require discrete protein domains, which include the DNA J, origin-binding and helicase domains. These facilitate viral replication by binding to cellular replication proteins, including Hsc70 (HSPA8), DNA polymerase, primase, topoisomerase I, and RPA (Figure 1) [17]. SV40 large T antigen also binds cellular growth regulators including the tumor suppressors pRb and p53, to promote cell growth and entry into S phase. Importantly, the predicted MCPyV large T antigen contains many of the features common to oncogenic polyomaviruses, including an LxCxE motif that may directly bind pRb.

There are two published MCPyV genome sequences, obtained from sequencing MCPyV DNA integrated into two MCC tumors. In both sequences, premature stop codons are predicted within the second exon of large T after the pRb- and Hsc70-binding domains but before the helicase domain required for viral replication. Such truncations were probably selected during tumor development to render the large T antigen protein inactive for viral DNA replication functions. If the large T antigen were intact, replication initiated at an integrated origin could lead to genomic instability and cell death. Indeed, in permissive cells transformed by SV40, the large T antigen is often mutated such that the viral replication functions (helicase activity) are inactive but some cell-cycle progression functions (pRb binding) are preserved [18,19]. Thus, the predicted protein products of the two truncated MCPyV large T antigens are likely to maintain pro-carcinogenic effects but have lost their cell-lethal effects in a fashion similar to the SV40 large T antigens found in virus-induced animal cancers.

Polyomavirus family members are known to have two other T antigen proteins with transforming ability. MCPyV is predicted to express a small T antigen that is able to bind to and inactivate protein phosphatase 2A (PP2A). A few polyomaviruses express an additional middle T antigen that binds Src and other signaling proteins. On the basis of the published sequences, it is unlikely that MCPyV encodes a middle T antigen.

Five polyomaviruses have been discovered in humans: BK, JC, WU, KI (all named after the patients or institutions where they were discovered) and now MCPyV. Serologic evidence of BK virus infection can be detected in nearly all humans and active BK infection causes nephritis in immunocompromised patients, particularly renal transplant recipients [20]. The JC virus also commonly infects humans and can cause progressive multifocal leukoencephalopathy in immunocompromised persons [21]. BK virus has been detected in prostate cancer and JC virus in a variety of lung, brain, colon and other tumor types [21]. However, in these cancers the BK and JC viruses have been found to be episomal and not integrated; thus, their contribution to carcinogenesis remains unclear. WU and KI viruses were recently discovered in nasopharyngeal secretions [22,23].

**Figure 1**

Alignment of key functional domains of MCPyV, JC, and SV40 large T antigens. At the top is a cartoon of the protein structure of the predicted MCPyV large T antigen, based on its homology to the well-characterized SV40 large T antigen (modified from [17]). Underneath are expanded alignments for the Hsc70-binding motif and the pRb-binding motif, comparing the putative MCPyV large T antigen with the JC and SV40 large T antigens. Importantly, as indicated by the bold underlines below the zoom-ins, both the HPDK Hsc70-binding motif and the LxCxEx Rb-binding motifs are preserved in the predicted MCPyV large T antigen. Noted on the cartoon are the locations of the premature stop (MCV350) and frameshift (MCV339) mutations of the two known MCPyV genomes [11]. These predicted truncated proteins potentially preserve some of the cell-cycle progression activities of the amino terminus of large T but prevent cell-lethal genomic instability related to the replicative functions of the carboxyl terminus.

Merkel cell polyomavirus is currently the only polyomavirus with demonstrated integration into human tumor DNA.

### Does MCPyV contribute to MCC pathogenesis?

Several significant observations suggest that MCPyV contributes to the development of a subset of MCC: it is present in a substantial portion of MCC tumors, viral integration appears to be an early event, large T antigen transcript is expressed in MCC tumors, and the MCPyV large T antigen predicted protein sequence shares key homology with the SV40 T antigen oncoprotein [11]. Furthermore, the strong epidemiologic links between immunosuppression and the incidence and severity of MCC support an infectious etiology.

In contrast, there are also arguments against an essential role for MCPyV in MCC oncogenesis. It appears that MCPyV infection is not necessary for the development of MCC; indeed, two out of ten reported MCC cases were MCPyV negative [11]. This distinguishes MCPyV from other cancer-causing viruses such as KSHV and human papillomavirus (HPV) where viral sequences are found in nearly 100% of tumors. The epidemiology of MCC can also argue against the important involvement of any virus in the disease. MCC incidence is strongly linked to sun exposure and MCC very rarely develops in persons with heavily pigmented skin. Furthermore, there have been no reports of clusters of MCC or of several people in the same family developing MCC, as might be expected were it due to a viral infection.

The discovery of MCPyV raises several interesting questions. It will be important to determine the prevalence of MCPyV in healthy individuals as well as to test other cancers for the presence of integrated MCPyV. Furthermore, it will be helpful to distinguish whether MCPyV infection is involved in tumor initiation and/or tumor maintenance, especially in regard to the viral T antigens. It will be especially important to determine whether viral proteins are continually expressed in MCC tumors, and if these viral genes are required for the ongoing survival of cancer cells. These studies will help guide efforts to design potential small-molecule drugs or anti-tumor vaccines.

For the moment, whether MCPyV promotes carcinogenesis in MCC or other cell types remains a captivating question. It is clear that the discovery of MCPyV by Chang and Moore and their team [11] has opened up important new avenues that should illuminate MCC biology and also that the DTS technique may be applicable to the discovery of other pathogens.

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